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A Common Genetic Basis in Sweet Corn Inbred Cr1 for Cross Sensitivity to Multiple Cytochrome P450-Metabolized Herbicides

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Nicosulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone are postemergence herbicides from different chemical families with different modes of action. An association between the sensitivity of sweet corn to these herbicides was observed when 143 $F_{3:4}$ families (F_4 plants) derived from of a cross between Cr1 (sensitive inbred) and Cr2 (tolerant inbred) were evaluated in greenhouse trials. The ratio of tolerant : segregating : sensitive families was not significantly different from a 3 : 2 : 3 ratio, which would be expected if a single gene conditioned herbicide response. Families cosegregated for responses to these herbicides. In field studies with 60 $F_{3:5}$ families in 2005 and 120 $F_{3:5}$ families in 2007, responses to these herbicides and foramsulfuron and primisulfuron were associated. Responses to bentazon in field trials were similar to the aforementioned herbicides for tolerant families, but differences were noted for families that were sensitive or segregated for responses to nicosulfuron, foramsulfuron, primisulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone. The gene(s) affecting herbicide sensitivity in Cr1 maps to the same region of chromosome 5S as a previously sequenced cytochrome P450 gene, where alleles previously designated *nsf1* and *ben1* were associated with sensitivity to nicosulfuron and bentazon and appear to be the result of a 392–base-pair insertion mutation. This work supports the hypothesis that a single recessive gene or closely linked genes in the sweet corn inbred Cr1 condition sensitivity to multiple cytochrome P450 enzyme-metabolized herbicides.
Nomenclature: Bentazon; carfentrazone; dicamba; diflufenzopyr; foramsulfuron; nicosulfuron; mesotrione; primisulfuron; sweet corn, *Zea mays* L.
Key words: Cytochrome P450, herbicide metabolism, herbicide selectivity, herbicide tolerance.

Sweet corn hybrids and inbreds are evaluated routinely for responses to herbicides. Certain hybrids and inbreds are severely injured by postemergence herbicides, including acetolactate synthase (ALS) –inhibiting nicosulfuron (Grey et al. 2000; O'Sullivan and Bouw 1998; O'Sullivan et al. 2000), foramsulfuron (Diebold et al. 2003), primisulfuron (Grey et al. 2000; O'Sullivan and Sikkema 2002), and rimsulfuron (O'Sullivan and Bouw 1998); 4-hydroxyphenylpyruvate-dioxygenase-inhibiting mesotrione (Masiunas et al. 2004; O'Sullivan et al. 2002; Williams et al. 2005), and photosystem II (site B) –inhibiting bentazon (Diebold et al. 2004). An association between sensitivity of sweet corn to nicosulfuron and mesotrione was observed among hybrids, inbreds, and $F_{2:3}$ families, i.e., F_3 plants planted ear-to-row from the F_2 plants on which seed was produced (Green and Williams 2004; Williams et al. 2005). Nicosulfuron-sensitive hybrids were more likely to be sensitive to mesotrione than nicosulfuron-tolerant hybrids and vice versa. This association was more evident among inbreds than among hybrids, presumably because inbreds are homozygous for genes affecting herbicide sensitivity, whereas hybrids could be homozygous or heterozygous for those genes. Segregation of F_2 families for response to nicosulfuron and mesotrione fit a 1 : 2 : 1 pattern, which is expected if sensitivity is conditioned by a single recessive gene. Some nicosulfuron- and mesotrione-sensitive inbred lines and F_2 families also appeared to be sensitive to dicamba plus diflufenzopyr (growth regulators) and carfentrazone (a protoporphyrinogen oxidase inhibitor) (Pataky et al. 2006).

Differences in rates of metabolic detoxification are the primary basis for differential sensitivity in corn and other plants to sulfonylurea herbicides (Barrett 1995; Green and Ulrich 1993; Harms et al. 1990; Hinz and Owen 1996). Plants that rapidly metabolize sulfonylurea herbicides are tolerant, whereas plants that metabolize these herbicides slowly are sensitive (Sweetser et al. 1982). Nicosulfuron is hydroxylated by cytochrome P450 enzymes in corn as a Phase I detoxification reaction (Kreuz et al. 1996). Cytochrome P450 enzyme activities in corn also metabolize herbicides in at least five other chemical families; however, the number of P450 enzymes involved and regulation of their levels of activity are not clearly understood (Barrett 1995, 2000; Boldt et al. 1992).

A growing body of evidence suggests that sensitivity of corn to multiple P450-metabolized herbicides is regulated by a single gene or a group of closely linked genes on the short arm of chromosome 5. Sensitivity of some corn inbreds and hybrids to herbicides such as bentazon, nicosulfuron, primisulfuron, and thifensulfuron has been reported as being conditioned by single recessive genes (Fleming et al. 1988; Green and Ulrich 1993; Harms et al. 1990; Kang 1993; Pataky et al. 2006; Widstrom and Dowler 1995). Kang (1993) proposed the gene designation *nsf1* for a single gene conditioning nicosulfuron sensitivity in the field corn inbred W703a. Bradshaw et al. (1994) reported that sensitivity of the dent corn inbred GA209 to bentazon was controlled by two recessive genes; i.e., tolerance was conditioned by duplicate, dominant, independent genes designated *Ben1* and *Ben2*. The *Ben1* gene conditioned tolerance to nicosulfuron and bentazon; whereas the *Ben2* gene conditioned tolerance only to bentazon (Barrett 1997). Barrett et al. (1997) observed that GA209 was sensitive to bentazon, chlorsulfuron, imazethapyr, nicosulfuron, and primisulfuron and slightly more sensitive to dicamba than B73, a bentazon- and nicosulfuron-tolerant line. Barrett et al. (1995) proposed that a few or even one P450 enzyme is primarily responsible for metabolism of these herbicides. Williams et al. (2006) recently used a map-based

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cloning approach to locate the *Nsf1* gene on the short arm of chromosome 5 and to sequence the dominant allele from a nicosulfuron-tolerant inbred B73. The *Nsf1* gene was one of four closely linked genes with homologies to cytochrome P450 genes, including the highly conserved heme-binding sequence FxxGxxxCxG found in most P450s. Nicosulfuron-sensitive inbreds GA209 and W703a contained a 392–base-pair insertion in the *Nsf1* sequence relative to B73. Thus it appears that this insertion results in a nonfunctional P450 allele and the *nsf1* and *ben1* alleles identified from W703a and GA209, respectively, are the same.

Previously, the sweet corn inbred Cr1[1] was observed to be sensitive to nicosulfuron, mesotrione, dicamba plus diflufenzopyr, carfentrazone, primisulfuron, rimsulfuron, foramsulfuron, and bentazon (Pataky et al. 2006; Williams et al. 2005). Sensitivity to each of these P450 enzyme-metabolized herbicides except bentazon was inherited as a single recessive gene, although intermediate phenotypic responses to some herbicides indicated that partial dominance or codominance may affect response to some of the herbicides under certain conditions (Pataky et al. 2006). Landi et al. (1989) has observed codominant responses of corn lines to chlorsulfuron. If a single gene in Cr1 is primarily responsible for regulation of P450 detoxification of multiple herbicides, phenotypic responses of lines derived from crosses of Cr1 and herbicide-tolerant inbreds should display a strong association, i.e., lines should cosegregate for responses to multiple herbicides.

A better understanding of the relationships between responses of sweet corn to postemergence herbicides will help plant breeders identify sensitive lines at early generations in breeding programs, and will assist herbicide manufacturers in preparing pesticide labels that adequately address the risks of using these chemicals on sweet corn. To avoid these risks, which include crop yield loss, certain herbicide labels presently advise applicators to contact local sales representatives regarding the sensitivity of specific sweet corn hybrids. A genetic understanding of crop sensitivity to individual herbicides does not necessarily require data on crop yield and may be obtained from knowledge of early-season crop response (Bradshaw et al. 1994; Kang 1993). The objective of this study was to determine if a common genetic basis in the sweet corn inbred Cr1 conditions sensitivity to multiple cytochrome P450-metabolized postemergence herbicides and to identify the chromosomal location of the gene responsible for this cross sensitivity.

Materials and Methods

Plant Materials. The F_1 hybrid was produced by crossing Cr1 and Cr2. Cr1 and Cr2 are white-kernel inbreds with the sugary enhancer endosperm mutation. Cr1 is sensitive and Cr2 is tolerant to multiple P450-metabolized postemergence herbicides. $F_{3:4}$ families were developed by self-pollinating F_1 plants ear-to-row for three generations. F_4 plants (i.e., progeny of the third self-pollinated generation) in $F_{3:4}$ families (i.e., a family consisting of F_4 plants all derived from the same F_3 ear parent) were evaluated in all greenhouse trials. $F_{3:5}$ families were produced by selfing and bulking seed from a minimum of eight F_4 plants from each F_3 family. F_5 plants (i.e., progeny of the fourth self-pollinated generation) bulked within F_3 families ($F_{3:5}$ families) were evaluated in field trials. Among six greenhouse and 16 field trials, the F_3 families were evaluated in 38 replicates, with a total of over 110,000 individual F_4 or F_5 plants being assessed for herbicide injury.

Greenhouse Trials. F_4 plants in 143 F_3 families were evaluated for responses to nicosulfuron,[2] mesotrione,[3] dicamba plus diflufenzopyr,[4] and carfentrazone[5] in greenhouse trials. Each herbicide treatment was a separate trial. Nicosulfuron and mesotrione trials were repeated. An experimental unit was approximately 30 plants per family in three rows (10 plants per row) in one half of a 30 × 60 × 7-cm flat. Flats contained a sterilized 1 : 1 : 1 mixture of soil, peat, and perlite supplemented with 14–14–14 Osmocote®[6] pellets. Families were arranged randomly within trials and two families were planted per flat. Each flat also included two plants each of the parental inbreds, Cr1 and Cr2, as controls. Each trial also included at least two experimental units each of Cr1, Cr2, and Cr1 × Cr2 as additional controls. Natural sunlight was supplemented with metal halide lamps for an intensity of 1,000 μmol m^{-2} s^{-1} at the plant surface for 14 h. Plants were watered as needed for healthy growth. The greenhouse was maintained at 24 ± 4 C during a 14-h day.

Commercial formulations of herbicides were applied when plants had three to four visible leaf collars. Two flats of plants were treated simultaneously in an herbicide spray chamber equipped with a flat-fan nozzle that delivered 187 L ha^{-1} of spray solution at 262 kPa. Nicosulfuron was applied at 35 g ai ha^{-1} with 0.25% (v/v) nonionic surfactant (NIS) and 2.5% (v/v) spray solution of 28% urea ammonium nitrate (UAN). Mesotrione was applied at 105 g ai ha^{-1} with 1% (v/v) crop oil concentrate (COC). Dicamba plus diflufenzopyr was applied at 210 g ai ha^{-1} ai plus 84 g ai ha^{-1}, respectively, with 0.25% (v/v) NIS and 2.5% (v/v) spray solution of 28% UAN. Carfentrazone was applied at 19 g ai ha^{-1} with 1% (v/v) COC.

For nicosulfuron, mesotrione, and dicamba plus diflufenzopyr, injury was either present or absent on individual plants, whereas all plants had some degree of injury to carfentrazone. Therefore, two rating systems were used to assess injury 7 to 10 d after herbicides were applied. For nicosulfuron, mesotrione, and dicamba plus diflufenzopyr, individual plants were rated visually as sensitive (i.e., those with symptoms of injury) or as tolerant (i.e., plants without injury symptoms). For carfentrazone, each three-row experimental unit of F_3 families was rated visually for percent leaf necrosis from 0 to 100%. In order to account for variation in injury among F_4 plants within a family, two individuals independently assigned each experimental unit two ratings. The four ratings were then averaged for each experimental unit. For nicosulfuron, mesotrione, and dicamba plus diflufenzopyr, F_3 families were classified as sensitive, segregating, or tolerant based on the percentage of injured F_4 plants per family. Families were classified as sensitive if 50% or more of the plants were injured, segregating if 12 to 49% of the plants were injured, and tolerant if less than 12% of the plants were injured. If herbicide response was conditioned by a single gene, the ratio of tolerant : sensitive F_3 plants in segregating families F_4 families would be expected to be 3 : 1. Thus, 12% was selected as the boundary between tolerant and segregating responses of families based on a chi-square goodness-of-fit test ($P > 0.10$, $n = 30$) for a 3 : 1 ratio of tolerant : sensitive F_3 plants. On the same basis, 50% was selected for the boundary

between segregating and sensitive families ($P > 0.001$, $n = 30$). Families were classified as sensitive to carfentrazone if the average leaf necrosis was 73% or higher, segregating if the average leaf necrosis was between 61% and 73%, and tolerant if average leaf necrosis was 61% or less.

Responses of the F_3 families were compared among the four herbicide treatments individually. Also, a cluster analysis was used to group families based on their responses to all four herbicide treatments. The FASTCLUS procedure of SAS (SAS 2000) sorted families into three to seven groups based on the percentage of plants per family that were injured by nicosulfuron, mesotrione, or dicamba plus diflufenzopyr, and the leaf necrosis for carfentrazone. Cosegregation was examined among all six possible pairs of individual herbicides and for the four pairs of individual herbicides and groupings based on the cluster analysis. A binomial test of proportions (i.e., *t* test) was used to test the hypothesis that the percentage of families with similar responses to both treatments in a pair (i.e., sensitive to both, segregating for both, or tolerant to both) was significantly greater than 34.4%, which would be expected if responses to each herbicide was conditioned by an independent dominant gene (i.e., 22/64 = (3/8)(3/8) + (2/8)(2/8) + (3/8)(3/8)). The hypothesis that the percentage of families with opposite responses to pairs of treatments (i.e., sensitive to one herbicide and tolerant to the other) was significantly less than 28.1% [i.e., 18/64 = (3/8)(3/8) + (3/8)(3/8)] also was tested. Also, ratios of sensitive, segregating, and tolerant families for each individual herbicide and for groupings based on the cluster analysis were tested by chi-square goodness of fit to a 3 : 2 : 3 ratio (tolerant : segregating : sensitive) that would be expected if herbicide response was controlled by a single gene with dominant gene action.

Field Trials. F_5 plants in F_3 families were evaluated for response to the four herbicides tested in the field trials. Only 60 F_3 families were tested in 2005 due to availability of seed. One hundred twenty F_3 families were tested in 2007. Foramsulfuron,[7] primisulfuron,[8] bromoxynil[9] (a photosystem II site B inhibitor), and sodium salt of bentazon[10] also were included in the field trials in order to observe responses of the families to two additional ALS-inhibiting herbicides (foramsulfuron and primisulfuron) and two photosystem II site B inhibitors (bromoxynil and bentazon). Trials were planted on May 5, 2005 and May 7, 2007 at the University of Illinois Crop Sciences Research and Education Center in Urbana, Illinois. Fields had been fertilized with 202 kg N ha^{-1} and a preemergence application of 2.2 kg atrazine ha^{-1} plus 1.8 kg *S*-metolachlor ha^{-1} was made for early-season weed control. Herbicide treatments were applied June 3, 2005 and June 11, 2007. Each herbicide treatment was evaluated in a separate trial that included two replicates of 60 or 120 F_3 families and four controls (Cr1, Cr2, Cr1 × Cr2, and a nicosulfuron-sensitive hybrid, DMC 20–38). The soil type was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudoll). In 2005, treatments were arranged in a modified split-plot design. Main plots were groups of four rows of families that were classified as sensitive, segregating, or tolerant in greenhouse trials. Subplots were 5.3-m rows of individual families with approximately 30 plants per row. In 2007 the experimental design was a randomized complete block with the experimental unit being an individual family with approximately 30 plants per row.

Commercial formulations of herbicides were applied when plants had three to four visible leaf collars. Nicosulfuron was applied at 35 g ha^{-1} with 1% (v/v) COC and 3.6% (v/v) spray solution of 28% UAN. Mesotrione was applied at 105 g ha^{-1} with 1% (v/v) COC and 2.5% (v/v) spray solution of 28% UAN. Dicamba plus diflufenzopyr was applied at 210 g ha^{-1} plus 84 g ha^{-1}, respectively, with 0.25% (v/v) NIS and 1.25% (v/v) spray solution of 28% UAN. Carfentrazone was applied at 19 g ha^{-1} with 1% (v/v) COC. The commercial formulation of foramsulfuron, which includes the safener isoxadifen-ethyl at a 1 : 1 ratio, was applied at 37 g ai ha^{-1} with 1.35% (v/v) methylated seed oil and 3.6% (v/v) spray solution of 28% UAN. Primisulfuron was applied at 40 g ai ha^{-1} with 1% (v/v) COC and 7.2% (v/v) spray solution of 28% UAN. Bromoxynil was applied at 420 g ai ha^{-1} (without adjuvants) and bentazon was applied at 1120 g ai ha^{-1} with 0.9% (v/v) COC and 3.6% (v/v) spray solution of 28% UAN. Herbicides were applied on the same day using a sprayer calibrated to deliver 130 L ha^{-1} of spray solution at 276 kPa.

Families were rated visually by two evaluators for symptoms of herbicide injury 7 to 14 d after application. In 2005, F_3 families were scored from 1 to 5 for each herbicide treatment except carfentrazone and bromoxynil, where 1 = tolerant families (no plants with symptoms of herbicide injury), 3 = segregating families (approximately 37.5% of plants displaying symptoms of herbicide injury) and 5 = sensitive families (all plants with symptoms of herbicide injury). Mean scores were used to classify families as tolerant (1 to 2), segregating (2.5 to 3.5) or sensitive (4 to 5). In 2007, individual F_5 plants were rated as sensitive or tolerant, and families were classified as tolerant, segregating, or sensitive based on percentage of sensitive plants as described previously for greenhouse trials. In the carfentrazone and bromoxynil trials, some injury occurred on all plants. Therefore, in both years, symptoms were rated independently by two evaluators for each family on a 1 to 5 scale, where 1 equaled less than 10% injury and 5 equaled more than 75% injury. Injury scores were averaged over evaluators and replicates for carfentrazone and bromoxynil.

Cosegregation of $F_{3:5}$ families was examined among all pairs of herbicide treatments as described above. Responses of families to each of the eight herbicide treatments in the field trials also were compared to the classification of families from the cluster analysis of responses in greenhouse trials.

Chromosomal Location of the Gene(s) Conditioning Cross Sensitivity. One hundred twenty-one of the $F_{3:4}$ families were fingerprinted at 80 polymorphic simple sequence repeat (SSR) marker loci covering most of the corn genome. A linkage map was generated based on a General Mills consensus map. The quantitative trait loci (QTL) analysis used family mean phenotypic responses to all herbicides based on the cluster analysis and family means for each of the four individual herbicides evaluated in greenhouse trials. A log-likelihood (LOD) threshold of 2.5 was chosen for declaring a putative QTL significant. The QTL position was determined at the LOD maxima in the region under consideration.

Results and Discussion

Greenhouse Trials. F_3 families segregated for response to all four herbicide treatments. The number of sensitive families

Table 1. Response of F_3 families to postemergence herbicide treatments in greenhouse trials and goodness-of-fit tests.

Response of family[a]	Nicosulfuron	Mesotrione	Dicamba + diflufenzopyr	Carfentrazone	Groups from clusters
	No. of families				
Sensitive	52	48	50	47	53
Segregating	45	48	41	48	43
Tolerant	44	47	49	44	47
χ^{2} [b]	4.2	5.6	1.4	6.8	2.3
Prob.	0.12	0.06	0.5	0.03	0.32

[a] For nicosulfuron, mesotrione, and dicamba plus diflufenzopyr, F_3 families were classified as sensitive when ≥ 50% of plants were injured, segregating if ≥ 12 to 49% of plants were injured, and tolerant if < 12% of plants were injured. For carfentrazone F_3 families were classified as sensitive if the leaf necrosis was ≥ 73%, segregating if necrosis was < 73% and > 61%, and tolerant if necrosis was < 61%.

[b] Chi-square goodness-of-fit test for a ratio of 3 : 2 : 3 families that were sensitive : segregating : tolerant, which would be expected if tolerance to herbicides was conditioned by a single, dominant gene.

ranged from 47 to 52, the number of segregating families ranged from 41 to 48, and the number of tolerant families ranged from 44 to 49 among the four herbicides (Table 1). Nearly 100% (284 of 285) of Cr2 plants were tolerant to all of the herbicides and 96% (255 of 266) of Cr1 plants were sensitive to all of the herbicides. All F_1 plants (Cr1 × Cr2) were tolerant to nicosulfuron, but some had intermediate responses with slight (< 10%) symptoms of injury to mesotrione and dicamba plus diflufenzopyr.

A 3 : 2 : 3 ratio of sensitive, segregating, and tolerant F_3 families would be expected if a single gene conditioned response to an herbicide. When the number of F_3 families with sensitive, segregating, and tolerant responses to each herbicide were compared to a 3 : 2 : 3 ratio by chi-square goodness-of-fit tests, the null hypothesis failed to be rejected ($P > 0.01$) for each herbicide tested in the greenhouse. Therefore, for each herbicide, alleles at a single locus appear to be primarily responsible for conditioning tolerant or sensitive responses. This result is consistent with previous research on inheritance of sensitivity to nicosulfuron and other herbicides (Green and Ulrich 1993; Kang 1993; Pataky et al. 2006; Widstrom and Dowler 1995). It also is in accordance with Barrett's hypothesis that a single cytochrome P450 enzyme in corn may be responsible for metabolism of multiple herbicides (Barrett 1995).

$F_{3:4}$ families cosegregated for responses to the four herbicides. For all six comparisons of pairs of herbicides, the proportion of families with the same response to two herbicides was significantly greater than 0.34, which would be expected if response to each herbicide was conditioned by a single, independent gene. The proportion of families with the same response to two herbicides ranged from 0.86 for the comparison of nicosulfuron and mesotrione to 0.60 for the comparison of dicamba plus diflufenzopyr and carfentrazone (Table 2). Similarly, for all six comparisons of pairs of herbicides, only one or none of the 143 $F_{3:4}$ families had opposite responses to two herbicides (i.e., sensitive to one herbicide and tolerant to another), which was significantly less than 28.1% (40 families), which would be expected if herbicide responses were conditioned by single, independent genes. Responses of F_3 families to dicamba plus diflufenzopyr and carfentrazone were more variable than responses to nicosulfuron and mesotrione, largely because symptoms of injury resulting from dicamba plus diflufenzopyr (subtle growth abnormalities) and carfentrazone (quantitative differences in leaf necrosis) were more difficult to assess than symptoms of nicosulfuron (dead or alive) and mesotrione (bleached or green leaves). Consequently, families that were tolerant or sensitive to an herbicide may have been classified as segregating and vice versa. Considering that only 96% of the plants of the sensitive inbred Cr1 were classified as sensitive, and some plants of the F_1 of Cr1 × Cr2 had intermediate responses, it seems probable that some F_3 families were misclassified. Alternatively, dissimilar responses of families to pairs of herbicides may represent recombination of very closely linked genes that condition responses to different herbicides. If dissimilar responses of families are due to recombination, approximately one-eighth of the recombinants would go undetected due to segregation among recombinant F_2 plants that produce F_3 families; approximately six-eighths would be fixed for response to one herbicide (sensitive or tolerant) and segregating for response to the other herbicide; and approximately one-eighth would have opposite responses (tolerant vs. sensitive) to the two herbicides. Among the six comparisons of pairs of herbicides in this study, 20 to 56 families had dissimilar responses (i.e., potential recombinants). Therefore, based on recombination, the number of families with opposite responses would have been expected to range from three to eight for any pair of herbicides. We never observed more than one family with opposite responses to any of the six pairs of herbicides. Thus, the associations among $F_{3:4}$ family responses to pairs of herbicides support the hypothesis that the same gene, or possibly very closely linked genes, are involved in conditioning sensitivity to all four herbicide treatments.

The cluster analysis of F_3 family responses to all four herbicides produced five groups that were defined easily. Group 1 included 47 families with tolerant responses (Table 3). Twenty families were tolerant to all four herbicides, 27 families were tolerant to three of the four herbicides, and

Table 2. Proportion of F_3 families with the same[a] or opposite[b] responses to four postemergence herbicide treatments in greenhouse trials.

	Nicosulfuron	Mesotrione	Dicamba + diflufenzopyr	Carfentrazone
Nicosulfuron	–	0.86[a]	0.75	0.70
Mesotrione	0[b]	–	0.74	0.66
Dicamba + diflufenzopyr	0.01	0.01	–	0.60
Carfentrazone	0.01	0.01	0.01	–

[a] Proportion of families with same responses to pairs of herbicides above the diagonal, i.e., tolerant to both, segregating for both or sensitive to both herbicides.

[b] Proportion of families with opposite responses to pairs of herbicides below the diagonal, i.e., tolerant to one and sensitive to the other herbicide.

Table 3. Groups of $F_{3:4}$ families based on cluster analysis of family response to four postemergence herbicide treatments in greenhouse trials.

			Mean and ranges of responses to herbicides for groups of families							
			Nicosulfuron		Mesotrione		Dicamba + diflufenzopyr		Carfentrazone	
Group	Response	n[a]	Mean	Range	Mean	Range	Mean	Range	Mean	Range
			% Sensitive plants per family						% Necrosis	
1	Tolerant	47	4	0–18	3	0–24	4	0–35	57	39–72
2	Segregating	36	29	9–48	25	6–55	23	0–48	62	50–78
3	Segregating[b]	7	22	0–41	23	0–37	96	76–100	70	61–81
4	Sensitive[c]	7	99	95–100	68	36–100	36	18–56	74	59–83
5	Sensitive	46	99	87–100	87	34–100	100	95–100	80	70–94

[a] n = number of $F_{3:4}$ families with sensitive, segregating, and tolerant responses.
[b] Segregating for response to nicosulfuron, mesotrione, and carfentrazone, but sensitive to dicamba plus diflufenzopyr.
[c] Sensitive to nicosulfuron, mesotrione, and carfentrazone but segregating for response to dicamba plus diflufenzopyr.

none of the 47 families were sensitive to any of the four herbicides. Group 2 included 36 families in which F_4 plants within families segregated for response to the four herbicides (Table 3). Twelve families were segregating for response to all four herbicides, and all 36 families segregated for responses to nicosulfuron and another herbicide or to mesotrione and another herbicide. Group 3 included seven families with predominantly segregating responses to nicosulfuron and mesotrione, but sensitive responses to dicamba plus diflufenzopyr (Table 3). Group 4 included seven families with sensitive responses to nicosulfuron and mesotrione or nicosulfuron and carfentrazone but segregating responses to dicamba plus diflufenzopyr (Table 3). Group 5 included 46 families with sensitive responses (Table 3). Thirty-six families were sensitive to all four herbicides, and eight families were sensitive to three of the four herbicides.

Responses of the F_3 families to individual herbicide treatments were compared to groups of families based on the cluster analysis (Table 4). Groups 2 and 3 were combined as segregating families. Groups 4 and 5 were combined as sensitive families. Thus, the cluster analysis produced 53 sensitive, 43 segregating, and 47 tolerant families. Compared to groupings from the cluster analysis, 94% (132 of 141) of the families had the same responses to nicosulfuron, 90% (129 of 143) had the same response to mesotrione, 80% (112 of 140) had the same response to dicamba plus diflufenzopyr, and 71% (98 of 139) had the same response to carfentrazone (Table 4). None of the families in the tolerant group were sensitive to individual herbicide treatments. Only one family in the sensitive group was tolerant to carfentrazone. When the number of families with sensitive, segregating, and tolerant responses based on cluster grouping were compared to a 3 : 2 : 3 ratio by chi-square goodness-of-fit tests, the null hypothesis failed to be rejected ($P = 0.32$) (Table 1). Thus, associations among responses of families based on the cluster analysis further support the hypothesis that the same or closely linked genes condition responses to all four herbicide treatments. Because the cluster analysis accounts for variation associated with evaluations of all of the individual herbicides, classification of families by this procedure should be less variable than classification by individual herbicides if in fact responses are conditioned by the same closely linked genes.

Field Trials. Field trials in 2005 and 2007 confirmed that, under natural environmental conditions, $F_{3:5}$ families from the cross of Cr1 and Cr2 had similar responses to the four herbicides tested in the greenhouse (nicosulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone) and to two additional herbicides (foramsulfuron and primisulfuron). Classifications of $F_{3:4}$ families from the cluster analysis of greenhouse trials were used as the benchmark for comparing responses of $F_{3:5}$ families in field trials. Early-season responses of $F_{3:5}$ families to seven of the eight herbicides (all except bentazon) were very similar to responses of families in greenhouse trials. For nicosulfuron, foramsulfuron, primisulfuron, mesotrione, and dicamba plus diflufenzopyr, 84 to 96% of the families evaluated in field trials in 2005 and 2007 had the same response as in greenhouse trials (Table 5). In 2005, all of the 21 families classified as tolerant in greenhouse trials were tolerant to nicosulfuron, foramsulfuron, primisulfuron, and bentazon; and 20 of the 21 families were tolerant to mesotrione and dicamba plus diflufenzopyr (Table 5). All of the 22 families classified as sensitive in greenhouse trials were sensitive to nicosulfuron, foramsulfuron, mesotrione, and dicamba plus diflufenzopyr; and 20 of the 22 families were sensitive to primisulfuron in 2005. Similar results were observed in 2007. All 46 of the families classified as sensitive in greenhouse trials were sensitive to

Table 4. Comparison of responses of $F_{3:4}$ families to individual herbicide treatments and groups of families formed by a cluster analysis of responses to all herbicides.

		Number of families, herbicide treatments, and responses											
		Nicosulfuron			Mesotrione			Dicamba + diflufenzopyr			Carfentrazone		
Groups based on cluster analysis	n[a]	Sen.[b]	Seg.[c]	Tol.[d]	Sen.	Seg.	Tol.	Sen.	Seg.	Tol.	Sen.	Seg.	Tol.
		No. of families											
Sensitive	53	52	0	0	47	6	0	45	7	0	44	7	1
Segregating	43	0	39	3	0	39	4	5	27	9	3	25	14
Tolerant	47	0	6	41	0	4	43	0	7	40	0	16	29

[a] Number of $F_{3:4}$ families with sensitive, segregating, and tolerant responses based on cluster analysis in Table 3.
[b] Sen. = sensitive response.
[c] Seg. = segregating response.
[d] Tol. = tolerant response.

Table 5. Responses of $F_{3:5}$ families to postemergence herbicide treatments in field trials.

Year	Response of family in greenhouse[a]	n[b]	Number of families with same response in field trials as in greenhouse trials					
			NICO[c]	FORA	PRIM	MESO	D + D	BENT
			No. of families in field trials					
2005	Sensitive	22	22	22	20	22	22	2
	Segregating	17	14	16	15	17	15	11
	Tolerant	21	21	21	21	20	20	21
2007	Sensitive	46	45	46	46	46	35	7
	Segregating	35	33	32	32	32	24	17
	Tolerant	39	31	30	31	35	35	38
			% Families with same response in greenhouse and field					
Combined			92	93	92	96	84	53

[a] Response of $F_{3:5}$ family to nicosulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone based on group formed by cluster analysis.
[b] Number of families with sensitive, segregating, or tolerant responses in greenhouse trials.
[c] Abbreviations: NICO, nicosulfuron; FORA, foramsulfuron; PRIM, primisulfuron; MESO, mesotrione; D + D, dicamba plus diflufenzopyr; CARF, carfentrazone; BROM, bromoxynil; BENT, bentazon.

foramsulfuron, primisulfuron, and mesotrione; and 45 of the 46 families were sensitive to nicosulfuron (Table 5). Fewer tolerant families were observed in the field in 2007, ranging from 30 to 35, compared to the 39 families classified as tolerant in greenhouse trials. In comparison of family responses in the greenhouse to carfentrazone and bromoxynil in the field, families classified as sensitive had 22 to 63% more injury than families classified as tolerant (data not shown).

Family responses to bentazon in field trials differed from family responses to nicosulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone in greenhouse trials. All but one of the families classified as tolerant in greenhouse trials also were tolerant to bentazon in field trials. However, only 9 of the 64 families classified as sensitive in greenhouse trials were sensitive to bentazon in the field trials in 2005 and 2007, and only 18 of 52 families classified as segregating in greenhouse trials were segregating for response to bentazon (Table 5). These results are consistent with the previous observation that tolerance to bentazon is conditioned by duplicate, dominant genes (Bradshaw et al. 1994).

Chromosomal Location of the Gene(s) Conditioning Cross Sensitivity. A single SSR marker, bnlg1382, on chromosome 5 (bin 5.01) was strongly associated (LOD score of 5.05) with phenotypic responses of $F_{3:4}$ and $F_{3:5}$ families to all of the herbicides. The maximal LOD score of 5.26 occurred between bnlg1382 and the marker locus mmc0151 (bin 5.0). Mean incidence (%) of sensitive plants in families homozygous for bnlg1382 marker alleles from Cr1 were 72, 83, 80, 66, and 64% for nicosulfuron, foramsulfuron, and primisulfuron, dicamba + diflufenzopyr, and mesotrione, respectively. Mean incidence (%) of sensitive plants in families homozygous for bnlg1382 marker alleles from Cr2 were 21, 30, 34, 24, and 22% for nicosulfuron, foramsulfuron, and primisulfuron, dicamba + diflufenzopyr, and mesotrione, respectively. Mean injury (1 to 9 scale) from carfentrazone was 6.6 and 5.3 for families homozygous for marker alleles from Cr1 and Cr2, respectively. The Maize Genetics and Genomics Database IBM2 2004 resolution map places bnlg1382 within 25 centimorgans of marker loci umc1766 and umc2036, which flank the *Nsf1* locus (Williams et al. 2006). Thus, the gene(s) in Cr1 that condition cross sensitivity to multiple P450-metabolized herbicides are closely linked to or an allele at the *Nsf1* locus.

A common genetic basis in the sweet corn inbred Cr1 for sensitivity to multiple cytochrome P450-metabolized herbicides was evident in greenhouse and field trials. F_3 families had similar responses to nicosulfuron, mesotrione, dicamba plus diflufenzopyr, and carfentrazone in greenhouse trials. The ratio of tolerant, segregating, and sensitive families for each herbicide was not different than 3 : 2 : 3, which was expected if herbicide response was conditioned by a single gene. Families appeared to cosegregate for response to these four herbicides based on higher-than-expected proportions of families with similar responses to pairs of herbicides and lower-than-expected proportions of families with opposite responses to pairs of herbicides. Because sensitivity to these herbicides appeared to be controlled by the same or very closely linked genes, a cluster analysis based on responses to all four herbicides was used to group families more accurately than classification of families based on responses to individual herbicides. Responses to nicosulfuron, foramsulfuron, primisulfuron, mesotrione, and dicamba plus diflufenzopyr in field trials were similar to and corroborated classification of families in greenhouse trials. Responses of families to bentazon and bromoxynil in field trials were different than responses to the other herbicides. Sensitivity to bentazon is conditioned by two recessive genes, *ben1* and *ben2* (Bradshaw et al. 1994). The *Ben1* gene has been associated with tolerance to multiple herbicides, and the *Ben2* gene was specifically associated with bentazon tolerance (Barrett et al. 1997).

The gene(s) affecting herbicide sensitivity in Cr1 map to the same region of chromosome 5S as a previously sequenced cytochrome P450 gene (Williams et al. 2006). An allele at this locus containing a 392–base-pair insertion mutation has previously been designated as *nsf1* and *ben1* genes, which have been associated with sensitivity to nicosulfuron, bentazon, and other P450-metabolized herbicides (Barrett et al. 1997; Fleming et al. 1988; Kang 1993). The gene(s) affecting herbicide sensitivity in Cr1 appear to be the same as or closely linked to the *nsf1*/*ben1* gene and may be present in other sweet corn inbreds and hybrids. If so, this could be the cause for sensitivity of sweet corn hybrids to several postemergence herbicides (Diebold et al. 2003, 2004; Grey et al. 2000; Masiunas et al. 2004; O'Sullivan and Bouw 1998; O'Sullivan and Sikkema 2002; O'Sullivan et al. 2000, 2002).

Sources of Materials

[1] Sweet corn inbreds, Crookham Company, P.O. Box 520, Cadwell, ID 83606-520.

[2] Nicosulfuron, Accent® Herbicide, DuPont.

[3] Mesotrione, Callisto® Herbicide, Syngenta.
[4] Dicamba plus diflufenzopyr, Distinct® Herbicide, BASF.
[5] Carfentrazone, Aim® EW Herbicide, FMC Corporation.
[6] Osmocote® pellets, Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.
[7] Foramsulfuron, Option® Corn Herbicide, Bayer CropScience.
[8] Primisulfuron, Beacon® Herbicide, Syngenta.
[9] Bromoxynil, Buctril® Herbicide, Bayer CropScience.
[10] Bentazon, Basagran® Herbicide, BASF.

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